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Gene-silencing suppressors for high-level production of the HIV-1 entry inhibitor griffithsin in Nicotiana benthamiana

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The exploration of emerging host organisms for the economic and efficient production of protein microbicides against HIV is urgently needed in resource-poor areas worldwide. In this study, the production of the novel HIV entry inhibitor candidate, griffithsin (GRFT), was investigated using Nicotiana benthamiana as the expression platform based on a non-viral vector. To increase the yield of recombinant GRFT, the RNA silencing defense mechanism of *N. benthamiana* was abolished by using three viral suppressors. A transient expression system was used by transferring the GRFT gene, which encodes 122 amino acids, under the control of the enhanced CaMV 35S promoter. The presence of correctly assembled GRFT in transgenic leaves was confirmed using immunoglobulin-specific sandwich ELISA. The data demonstrated that the use of three gene silencing suppressors allowed the highest accumulation of GRFT, with a yield of 400 µg g-1 fresh weight, and this amount was reduced to 287 µg g-1 after purification, representing a recovery of 71.75%. The analysis also showed that the ability of GRFT expressed in *N. benthamiana* to bind to glycoprotein 120 is close to that of the GRFT protein purified from E. coli. Whole-cell assays using purified GRFT showed that our purified GRFT was potently active against HIV. This study provides the first high-level production of the HIV-1 entry inhibitor griffithsin with a non-viral expression system and illustrates the robustness of the co-agroinfiltration expression system improved through the use of three gene silencing suppressors.

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